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Internal levels of plant growth regulators during *in vitro* culture of wild cherry (*Prunus avium* L.)

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Introduction

In vitro micropropagation of wild cherry is presently one of the main commercial ways to clonally propagate this species (Cornu and Boulay, 1986). In order to extend this technique to a large number of clones, it seems necessary to improve our knowledge of the behavior of the explants during the *in vitro* culture. Since plant growth regulators (PGR) play an important role in this technique (Margara, 1961), our attention was drawn to the effect of exogenous PGR on hormonal levels in the explants.

Materials and Methods

Wild cherry explants were cultured according to the procedure described by Riffaud and Cornu (1981). The micropropagation technique can be schematically divided into 3 stages: the multiplication stage, when axillary bud growth is promoted by an almost equal amount of indole-3-butyric acid (IBA, 4.9 μM) and benzyladenine (BA, 4.4 μM) in the culture medium; the elongation phase which was not studied; and the rooting phase, in which IBA (4.9 μM) alone promoted root formation.

Hormonal measurements were made during the multiplication and the rooting stages. For each measurement, 48 explants were divided into 3 parts: the apical part, including the apex *sensu stricto* and the youngest leaves inserted in the short internodes of the stem tip; the middle part of the explants, bearing the oldest leaves at the axis, whose axillary buds started to grow during multiplication treatment; and the basal part including the portion of the stem inserted into the culture medium, where roots were formed during the rooting stage.

For each series, explants were collected 0, 1, 2, 4 and 8 d after their transfer into fresh medium. Frozen samples were lyophilized and ground up with a ball mill. Analytical measurements were made following the procedure reported elsewhere (Label *et al.*, 1989). Techniques used were methanolic extraction, HPLC purification and fractionation, and immunological measurement (ELISA), (Leroux *et al.*, 1985; Maldiney *et al.*, 1986; Sotta *et al.*, 1987; Label and Sotta, 1988). ELISA measurements were repeated 5 times. Mean values are given.

Results

Morphological development

Under standard multiplication conditions (Fig. 1A, B and C), axillary buds located in

the middle part of the explants started to grow on d 4 and, after 4 wk of culture, the multiplication rate was 3. When IBA was omitted from this culture medium (Fig. 1B and C), no multiplication was observable; moreover, 82% of the explants were necrotic at the 4th wk. When BA was omitted from the standard multiplication medium (Fig. 1A), about 65% of the explants were rooted 4 wk after subculture.

Under standard rooting conditions (Fig. 1D), first root primordia were histologically observable on d 5 and, after 3 wk of culture, 80% of the explants had at least one root. When IBA was omitted from this culture medium (Fig. 1D), 3% of the explants were rooted at the 3rd wk.

Hormonal measurements

Endogenous hormonal levels are presented on the same unit scale in each figure (with and without exogenous PGR). This was done to point out some specta-

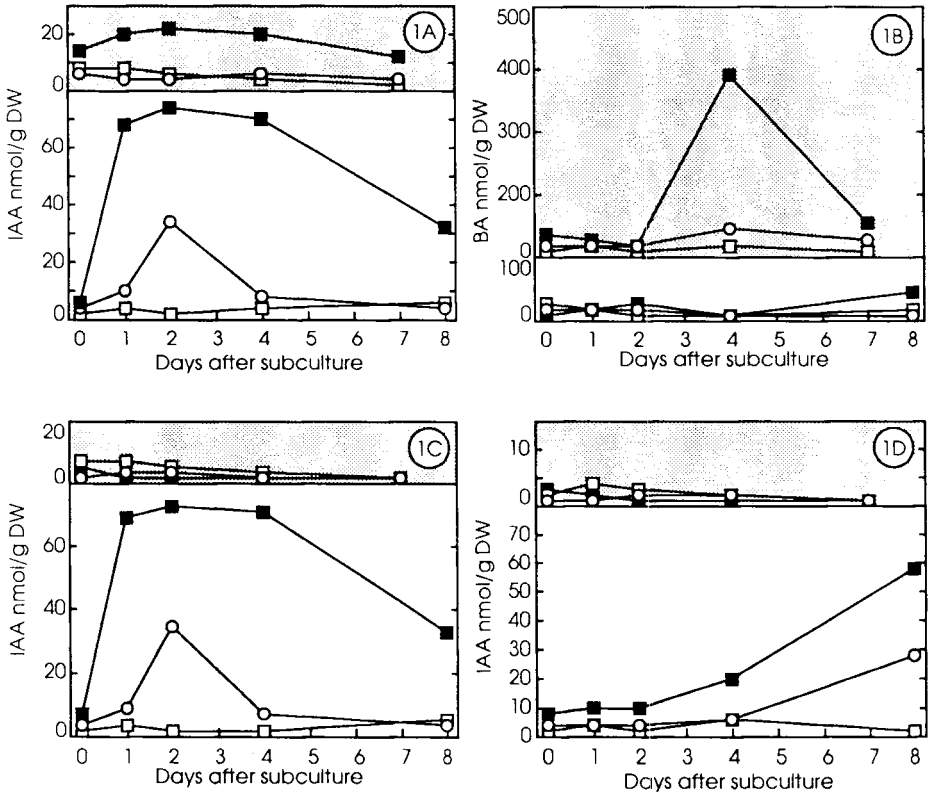

Fig. 1. Internal levels of IAA and BA during the first 8 d of culture. **A**: effect of exogenous BA on endogenous IAA content during multiplication; **B**: effect of exogenous IBA on internal BA content during multiplication; **C**: effect of exogenous IBA on endogenous IAA content during multiplication; **D**: effect of exogenous IBA on endogenous IAA content during rooting. □: apical part; ○: middle part; ■: basal part; ▨: without exogenous PGR in the culture medium; ■: with exogenous PGR in the culture medium (standard conditions).

cular differences between hormonal levels in the explants according to treatment. For instance, in Fig. 1C and D, without IBA in the culture medium, IAA levels were very low (grey background), but the apico-basal distribution of this hormone in the explants was, nevertheless, significant. Results are presented in nmol·g^{-1} DW; to approximately convert them into nmol·explant^{-1}, measurements given in the apical, middle and basal part will be multiplied by 3, 2 and 1, respectively.

Discussion and Conclusion

A relationship between IBA and endogenous IAA can be evoked. In each experiment, when explants were cultured with IBA (multiplication, rooting) in the culture medium, internal IAA was baso-apically distributed in the explants and IAA levels were 20–30 times higher than in explants cultured without IBA, where IAA is apico-basally distributed. Epstein and Lavée (1986) reported a transformation of IBA into IAA during *in vitro* culture of *Vitis vinifera* and *Olea europea.* The chemical pathway could be a β-oxidation of the butyric side chain, but the biochemical mechanism of this process remains unknown.

From the experiments run in the presence and in the absence of BA in the culture medium (Fig. 1B), we postulate that IBA might control the penetration of BA into the explants. We still have to investigate this point, and experiments using radiolabeled IBA and BA would be of great interest in this perspective.

The last result was not illustrated because of its strong clarity: when BA was present in the culture medium (with or without IBA), no natural cytokinins could be detected in the explants, whereas each time BA was removed from the culture medium, natural cytokinins could be quantified by the ELISA technique. Thus, BA had a clear depressive effect on endogenous natural cytokinin metabolism. Although BA metabolism is well known (Letham and Palni, 1983), results on the effect of BA on endogenous cytokinin metabolism have never been published. In the future, the intensity of this depressive effect and the biological activity of this synthetic PGR should be explored.

Acknowledgments

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